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EFFECTS OF LIVE YEAST, MONENSIN AND CONCENTRATE LEVEL IN DAIRY CATTLE DIETS ON GAS AND VOLATILE FATTY ACIDS PRODUCTION

By

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DECLARATION

I declare that **Effects of live yeast, monensin and concentrate level in dairy cattle diets on gas and volatile fatty acids production** is my own work and that all sources that I have used or quoted have been indicated and acknowledged by means of complete references and that this work has not been submitted before for any other degree at any other institution

BRENDA MOKATSE	**DATE**

DEDICATION

This work is dedicated to my late father Mr. Nhlanyane Johannes Mokatse whom I wish he was present to witness this achievement. May his soul rest in peace.

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LIST OF CONFERENCE PROCEDINGS

Papers presented in conference proceedings emanating from this study

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ABSTRACT

Two meta-analysis of effects of yeast culture and monensin in lactating dairy cows were first performed. Secondly, two experiments were performed to evaluate the effects of live yeast (LY) or monensin (M) or both (LY+M) on gas production and fermentation by rumen micro-organisms *in vitro* in low (40 %) and high (60 %) concentrate diets of dairy cows. Rumen contents were collected from one cannulated lactating Holstein cow. Gas production was measured from 0 to 48 h of incubation. Volatile fatty acids and ammonia nitrogen concentrations were measured after 48 h. Meta-analysis of monensin indicated decrease dry matter intake (DMI) and increasing milk yield, consequently improving feed efficiency. Meta-analysis of yeast culture did not show improved performance. These results highlighted the importance of the meta-analysis as a useful tool that can be employed to both summarize effects across studies and to investigate factors explaining potential heterogeneity of response. The batch fermentation showed that in high concentrate diet, M significantly increased ammonia nitrogen, decreased acetate, but tended to increase propionate concentration (7.9, 63.2, 18.6 vs. 6.3, 66.8, 14.2 mmol/l; respectively). Addition of LY increased acetate concentration (64.2 vs 66.8 mmol/l). Supplementation with M, LY and LY+M reduced total gas production by 37.1, 22.5 and 26.9 %, respectively, compared to control at 48 h. In low concentrate diet, M and LY+M decreased and increased acetate (60.1 and 69.7 vs. 7.1 mmol/l; respectively). Adding LY and LY+M produced 8.6 % less gas, and M treatment 3.4 % more gas than the control. Overall, at 48 h, high concentrate resulted in less gas than low concentrate diets. High concentrate diets showed increased ammonia (7.9 and 6.4 vs. 5.21 and 4.7 mmol/l) decreased acetate (63.0 and 63.2 vs. 67.0 and 69.7 mmol/l) with a tendency to increased propionate (18.6 and 18.9 vs. 14.6 and 14.1 mmol/l) compared to low concentrate in M and LY+M treated diets. These results indicate that the effects of M and LY on rumen fermentation are substrate dependent, the high-concentrate diet showing the greatest response.

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CHAPTER 1

1. INTRODUCTION

Feed additives are used as rumen manipulators to increase animal productivity. Direct fed microbial (DFM) have been used to improve animal performance. Although the results on effects of DFM are not consistent, they are reported to improve the establishment of beneficial gut micro-organisms and decrease the risk of ruminal acidosis (Ghorbani et al., 2002), increase milk production (Yoon and Stern, 1995) as well as stimulate cellulolytic and lactate-utilizing bacteria, increasing fibre digestion and flow of microbial protein to the small intestine (Newbold et al., 1996).

Yeast products and monensin are widely used to improve feed efficiency of ruminants, and there effects on the modulation of ruminal fermentations are well known (Van Nevel, 1991). Yeast cultures also have been shown to stimulate utilization of hydrogen by ruminal acetogenic bacteria (Chaucheyras et al., 1995).

It has also been suggested that yeast product and ionophores antibiotics such as monensin may be supplemented complementary to benefit ruminant production as they differ in their mode of action in the rumen. Ionophores have been used widely in the feedlot industry for many years, for the prevention of acidosis and bloat in feedlot, improve milk production, health and reproduction in dairy cows. Ionophores such as monensin play an important role in reducing the incidence of subclinical ketosis in dairy cows (Bagg, 1997). One positive effect of monensin supplementation is its ability to improve ruminant's performance, which is due to combination of other factors, and its increase in ruminal propionate production at the expense of ruminal acetate production (Bergen and Bates, 1984).

Although the effects of monensin and yeast on rumen fermentation are relatively well described, there is much less information available on their associative effects. A much better response on stabilizing feed intake, improved rumen fermentation can be expected as a result of combined positive effects of the two feed additives.

Effects of additives on ruminant performance can vary with the type of diet being fed (Bergen and Bates, 1984; Bonsembiante et al., 1984; Ruiz et al., 2001), and therefore it is important to evaluate the effects of feeding live yeast and monensin under different scenarios such as concentrate: forage ratio of the ruminant diet. Understanding and interpreting effects of the two additives would help producers and researchers to make decision on the choice.

Objectives of the study

The main objective of the study was to analyse effects of live yeast, monensin and concentrate level in dairy cattle diets on gas and volatile fatty acids production.

The specific objectives of the study are to:

1) determine the ffects of yeast culture and monensin on dry matter intake, milk yield and milk composition, body condition score (BCS), body weight (BW), energy corrected milk (ECM), feed efficiency (FE) and urinary nitrogen (UN)
2) determine the effects of high and low dietary forage levels with or without monensin live yeast supplementation on gas production, total and proportion of volatile fatty acids and ammonia nitrogen and rumen fermentation *in vitro*.

CHAPTER 2
2. LITERATURE REVIEW

2.1. Supplementation with monensin and yeast on rumen fermentation in dairy cows

2.1.1. Monensin in dairy cows

The use of monensin is one of the strategies that can be implemented to prevent negative energy balance and ketosis. Monensin is a carboxylic polyther ionophore produced by naturally occurring strains of *Streptomyces cinnamonensis* (Haney and Hoelm, 1967). Monensin has been reported to have a variety of beneficial effects in ruminants. Claims are for increased milk production, improved feed efficiency, control of subclinical and clinical ketosis and control of bloat (McGuffey et al., 2001). Feed cost is still the most important factor affecting profit margins in dairy herds. Therefore, any improvement in the conversion of feed to milk has a direct impact on profit margin of the dairy farm (Britt et al., 2003). In the USA, rumensin (active ingredient, monensin sodium), supplementation of dairy cows was recently approved, based on the claim of improved milk production efficiency (Shaver, 2005). Applied at the recommended dosage levels in fowls, monensin is practically not absorbed by the gastrointestinal tract and is not deposited in the muscles and internal organs (Biovet, 2000). Scientific data indicate that meat and milk produced from animals fed monensin is safe for human consumption. Likewise, monensin is biodegradable in manure and soil, and is not toxic for crop and plants (Ipharraguerre and Clark, 2005).

2.1.2. Mode of action of monensin in rumen

The primary mode of action of monensin in the rumen is bacteriostatic of certain bacterial populations. A secondary mode of action is the control of the protozoa that cause coccidiosis. Inhibition is achieved by disruption of ion transport in the microbial cells leading to excessive uptake of sodium ions and loss of potassium ion transport in the microbial cells (Russell and Strobel, 1989, Ipharraguerre and Clark, 2003). Gram negative bacteria are generally unaffected

by monensin as they have a complex outer membrane that is impermeable to larger molecules. Gram positive bacteria generally lack potassium, the complex outer membrane structure and so are susceptible to the inhibitory effects of monensin (Bagg, 1977; Iphrraguerre and Clark, 2003). Monensin inhibits organisms that produce hydrogen, a precursor for methane synthesis. Methanogenic bacteria are not affected by monensin directly (Russell and Strobel, 1989). Monensin inhibits lactate producing organisms (Russell and Strobel, 1989).

It is the general belief that monensin exerts many of its effects by modifying the composition of microbial populations in rumen (Dawson, 2005). However, monensin has also been shown to control microbial activity in the small intestine (Parker and Armstrong 1987). Intestinal tissue is highly metabolically active and has a high cell turnover rate. Supplementation with monensin reduces the gut turnover by controlling microbial activity and thus decreases the requirement of gut tissues for essential nutrients and makes more nutrients available for productive purposes. This may be another mode of action whereby monensin increases the feed efficiency of ruminant animals.

2.1.3. Effects of feeding monensin on metabolic disorder

During transition the dairy cow undergoes many metabolic changes in preparation for parturition and lactation. Decreased intake (Grummer, 1993), high glucose requirements of the foetus and the mammary gland (Bell, 1995), and decreased sensitivity of adipose tissue to insulin often result in hypoglycaemia and lipolysis in the periparturient dairy cow. The combination of these metabolic events sets the stage for postparturient metabolic diseases such as ketosis and fatty liver. If glucose supply is increased or even maintained during this time, the need for adipose tissue mobilization is decreased and subsequent incidences of metabolic diseases may be decreased.

Increased rumen propionic acid improves gluconeogenesis (Schelling, 1984). The reported health benefits of administrating ionophores of dairy cattle include bloat prevention and a reduction in the incidence of subclinical ketosis and associated clinical disease. A controlled released capsule (CRC) containing monensin has been found to help prevent pasture bloat in dairy cattle in

several studies conducted in Australia and New Zealand (Sauer et al., 1989; Lynch et al., 1990; Cameron and Malmo, 1993, Lowe et al., 1991).

It was recognised that many of the metabolic disorders afflicting cows during the periparturient period are interrelated in their occurrence and are related to the diet fed during prepartum period (Curtis et al., 1985). They have determined that increase energy content of the diet fed during the prepatum period was associated with decreased incidence of displaced abomasum and that increased protein content of the diet was associated with decreased incidence of retained placenta and ketosis (Curtis et al., 1985).

Monensin an ionophore that increases the ratio of propionate to acetate produced within the rumen (Van Nevel and Demeyer, 1997), decreases ruminal methane production (Russell and Strobe, 1989) and decreases ruminal protein degradation (Bergen and Bates, 1984). Ionophores benefit gram-negative ruminal bacteria at the expense of gram positive bacteria, resulting in a shift from lactate to propionate producing- organisms, with the effect of a lower risk of rumen acidosis (Goff and Horst, 1997). High producing ruminants, such as dairy cows, often suffer from digestive disorders related to lack of glucogenic precursors (Ipharraguerre and Clark, 2003), which is due to the rapid change in energy required by the animal between late gestation and early lactation. Monensin enhances propionate production which is major substrate for glucose production in the liver (Ipharraguerre and Clark, 2003). Supplementation with monensin, therefore, has led to a decrease in occurrence of nutrition related diseases such as ketosis and displaced abomasum (Duffield et al., 2002).

2.1.4. Monensin and energy metabolism in dairy cows

Monensin exerts its many effects by shifting the microbial populations in the rumen (Bergen and Bates, 1984), selectively inhibiting gram positive bacteria rather than gram negative bacteria because of differences in bacterial cell wall structure. Monensin changes the ratio of VFA in the rumen, increasing propionic acid and acetic acid (Richardson et al., 1976).

Monensin supplementation has decreased methane production in *in vitro* fermentations with rumen fluid (Russell and Strobel, 1988, Sullivan and Martin, 1999) as well as *in vivo* with dairy cows (Odongo et al., 2007). Approximately 12% of the animals feed energy may be lost to methane production (Russell and Stobel, 1989). Monensin supplementation has shown to decrease the ratio of acetate to propionate (Sullivan and Martin, 1999; Mutsvanga et al., 2002). Propionate provides more energy to the animal for productive purposes (Russell and Strobel, 1989). Increase in propionate lead to more glucose (Ipharraguerre and Clark, 2003; Breoderick, 2004) which is precursor of lactose and essential for milk synthesis (McGuffey et al., 2001).

2.1.5. Performance of dairy cows fed monensin

Supplementation with monensin is consistently reported (Beckett et al., 1998; Mutsvangwa et al., 2002). Monensin increased milk production by 0.75 L/d and tended to increase milk fat and protein yields (Beckett et al., 1998). There are numerous reports of improved feed efficiency in ruminants supplemented with monensin, either by animals decreasing feed intake and maintaining production or increases in production without any increase in feed intake (Daenicke et al., 1982.,. Phipps et al., 2000) reported that efficiency of milk production was increase by 5%, with monensin. There are also reports that monensin supplemented cows have a higher intake under conditions of sub-acute ruminal acidosis (Mutsvangwa et al., 2002). This is probably related to decreased lactate production with monensin. There are fairly consistent reports that monensin depresses microbial protein production due to its antimicrobial effect on the rumen (McGuffey et al., 2001). In some cases monensin has led to a depression in milk protein percentage (Broderick, 2004, Odongo et al., 2007). Depression in fibre digestion *in vitro* was also reported (McGuffey et al., 2001). A common effect of monensin is depressed by feed intake and this may be related to a decreased in rate of digestion of fibrous materials in the rumen. Enhanced propionate to acetate ratio has led to the depression of milk fat content due to the lack of lipogenic precursors (Mutsvangwa et al., 2002, Odongo et al., 2007). Monensin improves both energy and protein status of the animal, while supplementation with yeast culture may reduce the impact of monensin on the rumen bacterial population and animal feed intake. Monensin reduces the incidence of rumen acidosis through inhibition of lactate-producing organisms (Dawson,

2005). It seems that yeast culture may also help alleviating rumen acidosis by stimulating organisms that utilize lactate in the rumen (Callaway and Martin, 1997).

It was reported that body condition score (BCS) had an impact on milk production response. Cows classified as thin (BCS < 3.0) at three weeks before calving had no significant milk production response in the first 90 days after calving to monensin while cow's classified with good BCS (3.25 to 3.75) had a significant increase in milk yield 0.85 kg, and heavy cows (> 4.0 BCS) increased milk by 1.2 kg (Ipharraguerre and Clark, 2003).

Monensin increased milk production by 0.9 kg/cow/day in cows less than 150 days in milk, (Bergen and Bates, 1984). Most studies reported an effect of monensin on milk production stated an increase impact between o.4 and 2.8kg of milk/cow monensin improved milk-production efficiency by 1.8 % to 3.9% when fed throughout lactation and the dry period (Phipps et al., 2000).

2.2. Yeast culture in dairy cows

2.2.1.. Mode of action of yeast culture in the rumen

It appears that stimulation of rumen bacteria is central to the mode of action where yeast culture stimulates rumen fermentation. The exact mechanism by which the yeast culture stimulates rumen bacteria remains unclear (Dawson, 2000). It may be due to removal of oxygen from the rumen environment or due to some unidentified growth factor provided by the metabolically active yeast cells (Dawson, 1993). Selective stimulation of lactate utilizing bacteria (Callaway and Martin, 1997) may result in stabilization of rumen pH. The stabilized pH may further stimulate the growth of the pH sensitive bacterial populations of the rumen and provide a suitable environment for fibre digestion.

Bacterial are predominantly responsible for digestion in the rumen and it follows that high bacterial numbers increase the animal's digestion rate and therefore its digestive capacity. Increased rate of digestion of feed results in additional nutrients available to the animal as well as making more space in the rumen resulting in increased dry matter intake (DMI). Higher bacteria

numbers also result in additional microbial protein available to the animal for digestion in the small intestine. The final results are that more nutrients are available for the ruminant for production purposes (Figure 1).

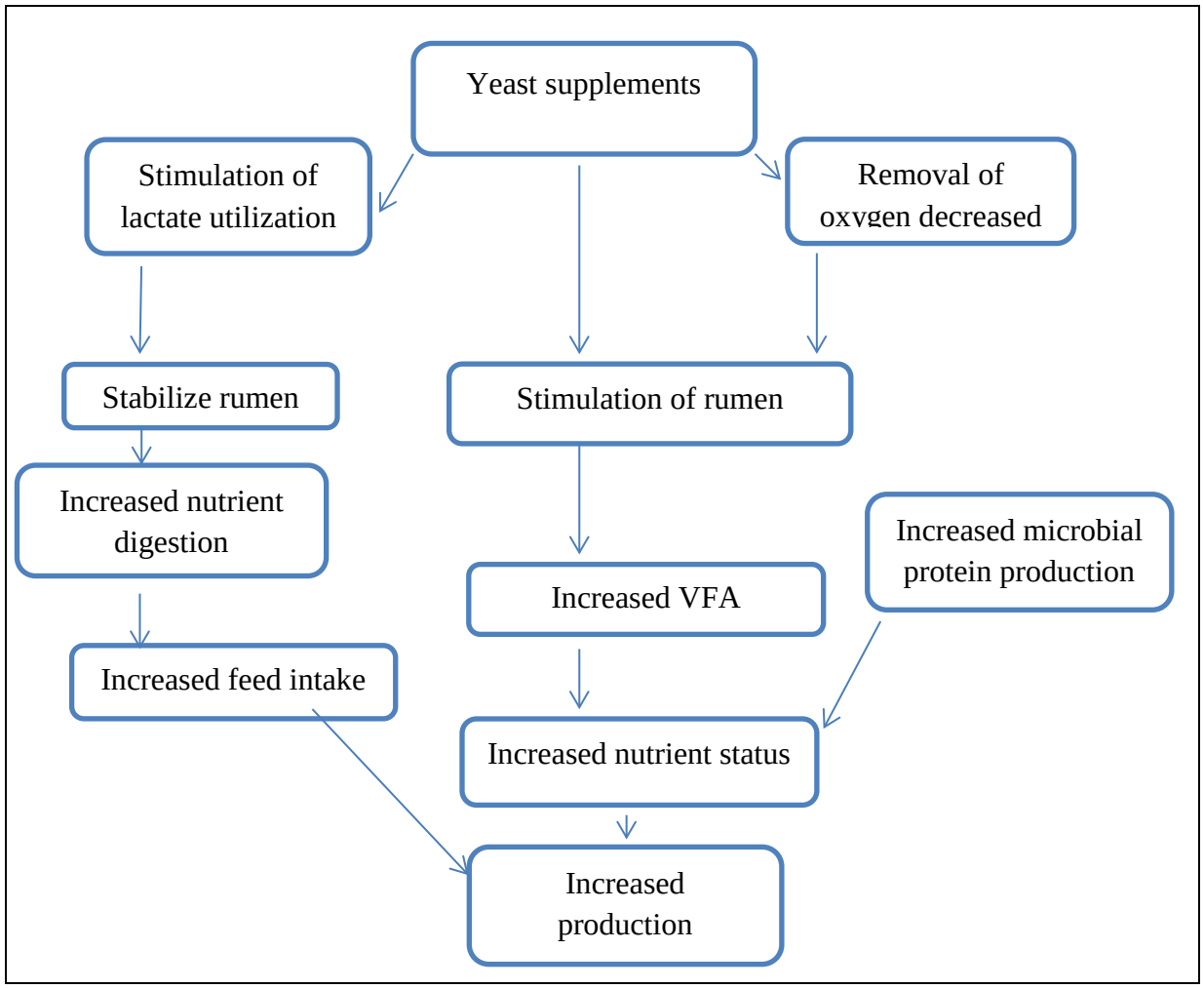

Figure 1: Schematic representation of an attempt to bring together the possible mode of action where yeast supplementation stimulates animal production. Source: Wallace, 1994

- **Oxygen scavenging**

Most of bacterial species that are responsible for the ruminal feed fermentation are strictly anaerobic. Oxygen entering the rumen with ingested feed and water increases the redox potential of the rumen inhibiting strict anaerobes (William and Newbold, 1990). Previous reports have

suggested that the cause of stimulatory effect of yeast culture on rumen fermentation may be their rapidly metabolism of oxygen entered into the rumen. This situation reduces the redox potential of the rumen, which create a more favourable growth condition for aerobic bacterial (Wallace, 1994; Miller-Webster et al., 2002) stimulating the strict anaerobic bacterial species.

- **Modulation of rumen pH**

Ruminant diets usually contain rapidly degradable carbohydrates in the form of starch from grains such as maize. These feed ingredients are fermented rapidly in the rumen and can lead to accumulation of fermentation intermediates such as lactate causing the pH of the rumen to decrease. The depression rumen pH inhibits the growth of many beneficial bacteria species in the rumen and is also strongly inhibitory to those organisms responsible for ruminal fibre digestion.

Reports indicate a decrease in total and peak lactate production as a result of yeast culture supplementation (Erasmus et al., 1992; Lila et al., 2004). Addition of yeast culture has also shown to stimulate the growth of major bacteria that utilise lactate *in vitro* (Callaway and Martin, 1997). Reports also indicate that yeast supplementation increases rumen pH (William et al., 1991; Beauchemin et al.; 2003, Sawant et al., 2005). Acid sensitive bacterial species, which fermentate feed ingredients in the rumen are stimulated by increased pH as result of decreased rumen lactic acid concentration.

- **Increased production of VFA**

Yeast culture cause increases in the total VFA produced in the rumen fermentation (Miller-Webster et al., 2002; Lila et al., 2004). Volatile fatty acids are main source of energy for ruminants (Miller-Webster et al., 2002; Lila et al., 2004).

- **Altered proportions of VFA**

Reports have shown that adding yeast culture depresses acetate to propionate ratio *in vitro* (Dawson et al., 1990; William et al., 1991; Miller-Webster et al.; 2002, Lila et al., 2004) and in *in vivo* (Williams et al., 1991). This stimulates animal production because propionate provides more energy to the animal than acetate.

2.2.2. Types of yeast product used in ruminant feeding

Aspergillus oryzae* and *Saccharomyces cerevisaie

Fungal supplements improve dry matter intake (DMI), milk production, milk composition and body weight (BW) gain of dairy cows. Improvements in performance have been attributed to increase numbers of ruminal cellulolytic bacteria, improvements in ruminal fibre degradation and changes in ruminal volatile fatty acid (VFA) (Kung et al., 1997). Yeast may also provide growth factors such as malate, to bacteria but utilize lactate that in turn may moderate changes in ruminal pH. Yeast culture has also shown to stimulate utilization of hydrogen by ruminal acetogenic bacteria. However supplemental yeast has not always altered ruminal metabolism or improved animal performance (Kung et al., 1997).

Freeze- dried *Saccharomyces cerevisiae*

Dietary supplements of yeast culture based on freeze dried *Saccharomyces cerevisiae* have been reported to improve health and productivity of ruminants. In comparison with antimicrobial agents, yeast cultures offer a natural alternative to manipulate animal performance. They have been shown to improve feed intake (Phillips and von Tungeln, 1985; Harris and Lobo, 1988) milk production (Harris and Webb, 1990; Piva et al., 1993; Kung et al., 1997; Dann et al., 2000) and weight gain (Phillips and von Tungeln, 1985; Hughes, 1988). However yeast cultures have not been found to alter ruminal metabolism or improve animal performance in all cases (Arambel and Kent, 1990; Chademana and Offer 1990; Williams et al., 1991; Cabrera et al., 2000; Arcos-Garcia et al., 2000).

Active dried yeast

Yeast products based on *Saccharamyces cerevisiae* are increasingly being used in ruminant diets to improve animal performance (Robinson and Erasmus, 2009). Numerous commercial products are available and these vary widely in the strains of *Saccharomyces cerevisiae* used and the number and viability of the yeast present. Host and dietary interactions may also alter the efficacy of some products. Consequently animal responses to yeast supplementation of diets can be variable. One potential mode of action of *S. cerevisiae* is to scavenge oxygen within the rumen creating a more anaerobic environment, which is required by ruminal microorganisms

(Newbold et al., 1996). *Saccharamyces cerevisiae* is thought to provide growth factors including organic acids, vitamin B and amino acids that stimulate microbial growth in the rumen, thereby indirectly stabilizing ruminal pH (Chaucheyras-Durand et al., 2008).

2.2.3. Ruminal effects of feeding yeast

Most dietary compounds entering the rumen are degraded by numerous aerobic microorganisms mainly bacteria and protozoa present in the rumen fluid. Rumen ecosystem plays a key role in ruminants' responses to diet .One of the consequences of feeding high concentrate diets is the occurrence of subclinical ruminal acidosis (rumen pH< 6.25) (Sauvant et al.,1999). Low pH in the rumen over a long period inhibits intake (Fulton et al., 1979; Owens et al., 1998).

The most consistently reported effect of yeast culture supplementation is an increase in the number of total anaerobic and cellulytic bacteria (Newbold et al., 1995) reported a more than 35% increase in total anaerobic and cellutytic bacteria in yeast supplemented rumen simulating cultures (Dawson et al., 1990) reported 5-40 fold increases in the concentration of cellulolytic bacteria in the rumen stimulating cultures supplemented with yeast culture. There are reports of changes in the concentrations of fermentation products (Miller-Webster et al., 2002) reported an increase in total VFA concentration in rumen simulating cultures supplemented with yeast culture (Miller-Webster et al., 2002) also reported the increase in molar proportion of propionate and decrease in the proportion of acetate in yeast supplemented rumen simulating cultures (Dawson et al., 1990) reported no significant change in the relative concentrations of VFA in rumen simulating cultures supplemented with yeast culture (Newbold et al., 1995) reported no change in the daily output of fermentation end product in response to yeast culture supplementation (Miller-Webster et al., 2002) reported an increase in the ammonia concentration in yeast supplemented rumen simulating culture while (Dawson et al., 1990) reported the significant change in the concentration of ammonia in yeast supplemented simulator. (Dawson et al., 1990; Newbold et al., 1995) reported no significant change in the pH of yeast supplemented continuous culture. Miller-Webster et al., (2002) reported an increase in dry matter digestibility in rumen simulating cultures supplemented with yeast. Newbold et al. (1995) reported no difference in dry matter digestibility in yeast supplemented rumen simulating cultures. Miller-

Webster et al., (2002) reported an increase in protein digestibility in yeast supplemented rumen simulating cultures.

2.2.4. Benefits of feeding yeast to dairy cows

It has been reported that the increase in dry matter and fibre digestibility in response to yeast supplementation (Lila et al., 2004). Increase in digestion of feed will lead to more nutrients being available to the animal and thus better animal production. However, other researchers reported no change in diet digestibility (Putnam et al., 1997)

Rate of digestion

There have been reports that yeast supplementation in ruminants may increase the rate of fibre digestion (William et al., 1991; Newbold et al., 1995; Lila et al., 2004). There is a general agreement in the literature that the effect of yeast on digestion, particularly fibre digestion, is on the rate rather than the extent of digestion. Increase in the rate of feed digestion may lead to a greater quantity of nutrients available for production. In addition it will increase the rate of emptying of the rumen. This may in turn increase DMI.

Dry matter intake (DMI)

Although DMI is not an estimate of digestibility, but it impacts digestibility directly and because it is thought that an increase in the rate of digestion may in turn increase the emptying rate of the rumen and therefore increase animal DMI (Wallace, 1994). Many researchers have reported an increase in DMI of ruminants fed yeast culture (William et al., 1991; Erasmus et al., 1992; Putnam et al., 1997). Increase feed intake will lead to increased productive output of animals by providing more nutrients to the animal. Other researchers reported no effects of yeast culture supplementation on DMI (Erdman et al., 1989; Kung et al., 1997).

2.2.5. Animal health and yeast culture supplementation

Response to yeast supplementation may vary according to the conditions of the rumen and environment. For example, if the average rumen pH of a dairy herd is low, then yeast

supplementation may have an effect in increasing the rumen pH and stabilizing the rumen fermentation. However, if the rumen pH of the herd is already at an optimum it is unlikely that yeast will have any effect on it (Williams et al., 1991). Newbold et al. (1995) suggested that the activities of the microbial population are increased by yeast supplementation and may improve nutrient status, particularly that of protein. However, it was pointed out that supplementation will have negligible effect in this regard if protein or the nutrient in question is not limiting in the first place. There may be variation in the response due the other parameters such as stage of lactation (Wohlt et al., 1991; Kung et al., 1997) or the way the yeast is presented (e.g. In an adlib ration or once daily top dressed on the feed). Therefore, the response to supplementation with yeast culture may be more likely when animals are fed a poor quality diet or when fermentation is disturbed (Dawson et al., 1990).

2.3. In vitro methods to estimate nutrient degradation

The *in vitro* dry matter digestibility method has been extensively used to evaluate the nutritional value of ruminant feeds. Tilly and Terry (1963) method has been largely used to analyse feedstuffs and has the most accurate and practical laboratory method available for predicting the digestibility data for ruminants (Goldman et al., 1987). The method has been modified and adapted for starch feedstuff analysis (Aufrere and Michalet- Doreau, 1988), and various workers have improved its accuracy of prediction. Different dilution buffers for the rumen liquor have been developed to adjust the pH of the inoculum (Grant and Mertens, 1992). Many laboratories have proven the method to be simple, highly repeatable and reproducible (Tilly and Terry, 1963; Goldman et al., 1987; Aufrere and Michalet-Doreau, 1988).

The rate and extents of ruminal degradation of feed protein are required in a number of systems of ruminant ration formulation. Lack of reliable data on protein degradation can cause dairy farmers to under or over feed protein to their cattle. To avoid problems due to either error, routine methods that are both accurate and rapid are needed to allow timely characterization of protein degradation of common feeds. A number of years have been devoted to develop an inhibitor *in vitro* method for assessing protein degradation (Broderick, 1978).

Various *in vitro* techniques have been used in the past as alternatives in the in Sacco method these consist of the use of rumen fluid, buffer, chemical solvents or commercial enzymes. Another technique uses the gas production (GP) system as an indirect measure of the *in vitro* digestion. The focus of the discussion is on the *in vitro* methods using the rumen fluid. *In vitro* techniques using rumen fluid are considered as methods for routine screening of feedstuffs due to their higher correlation with the *in vivo* digestibility (Holden, 1999).In addition, they are cheaper, easier and faster than the *in vivo* and *in Sacco* methods and these techniques offer the possibility of analysing both the residue and metabolites of microbial degradation. Furthermore, they allow control over various factors that alter the feed degradation (microbial, animal, environment) and, therefore provide uniform characterisation of feed for DM (dry matter) and protein degradation (Mohamed and Chaudry, 2008). The *in vitro* techniques were developed as alternatives for in *Sacco* method to study the ruminal degradation of feeds, but they are still unable to remove the need to use fistulated animals to collect rumen fluid.

All *in vitro* techniques currently in use (gas production system and ANKOM technique) are adapted from method described by Tilley and Terry (1963). This method consists in its first stage (as in the rumen) of incubating feed sample at 39 ºC in the rumen fluid, which is diluted with buffer solution similar in characteristics to saliva and saturated carbon dioxide (CO_2) to maintain anaerobic conditions. After 48 hours, the incubation is stopped and the incubation mixture filtered. The filtered residues are subsequently incubated in its second stage (as in the lower digestive tract) for another 48 hours with pepsin-HCI to remove un-degraded plant cell matter and microbial protein (Beever and Mould, 2000). The two–stage technique has still an inconvenient to use donor animals for rumen fluid. In addition, it only provides an end point measurement of digestion but not any information about the kenetic of digestion (Theodorou et al., 1994). To improve the post rumen digestibility, Goering and Van Soet, (1970) introduced the treatment of residues with the NDF solution.

In vitro methods involving the GP system consists of the measurement of the volume of gas production by fermenting feedstuffs using rumen fluid from fistiluted ruminant and buffer solution (Menke et al., 1979; Krishnamoorthy et al., 2005). These techniques involve collection and measuring of gas, the use of calibrated syringes (Menke et al., 1979) and pressure tranducer

(Theopdorou et al., 1994) in order to computerise gas monitoring devices (Pell and Schofield, 1993). The advantage of the automated gas production system is of high accuracy and reduction and the labour input. However, this option does not allow easy manipulations of large numbers of samples and ids expensive when compared to the manual method (Mohamed and Chauldry, 2008). According to Pell and Schofield (1993), the gas is produced from both soluble and insoluble metabolic energy sources. The in vitro GP intends to measure the potential conversion of different nutrient fractions (monosaccharaides, polysaccharides, pectin, starch, cellulose and hemicellulose) to CO_2, VFA and CH4. Many factors are reviewed by Mohamed and Chauldry, (2008) are likely to affect the accuracy of the GP technique.

These include: sample characteristics, buffer composition, ratio of rumen inoculum and buffer solution, prevailing pH and temperature, atmospheric pressure and stirring. Despite its poor correlation to the *in vitro* true digestibility (Getachew et al., 2004), the GP system is widely used due to its potential to accommodate large numbers of samples. It is also cheap, less time consuming and allows accuracy over experimental conditions than the in vivo trials (Getachew et al., 1998). High correlations between GP and NDF disappearance, r 2=.95 (Prasad et al., 1994) have been reported. Although the GP system is suitable to screen large numbers of feedstuffs or treatment by giving information on rate and extent of fermentation, it does not provide direct information of both the rate and extend of feed degradation or the quantity of end-products fermentation (VFA and MPS) available to the animal (Mauricio et al., 2001).

An ANKOM incubator and fibre apparatus developed by ANKOM Technology Corp. (Fairport, NY, USA) were introduced to improve the estimation of the *in vitro* true digestibility. The method consists of digesting forage samples into filter bags in suspension in the mixture of buffered solution and rumen fluid for the different periods of time, within rotating digestive jars in an insulated incubator (DAISYII incubator).

Besides being highly correlated to the in situ method (Spanghero et al., 2003), the filter bag technique is efficient to determine the rate and extent of degradation of feedstuffs (Holden, 1999). In addition, it reduces labour input as the technique prevents the filtration of residues in the estimations of *in vitro* digestibility (Cherney, 2000). Furthermore large numbers of feeds,

different forages, grain and mixed feeds can be incubated together in a single digestion jar. The DAISYII technique is seen as a rapid and convenient tool to evaluate *in vitro* digestibility of feeds in ruminant system.

2.4. Use of meta-analysis

Definitions and objectives of meta-analyses

Meta-analyses use objective, scientific methods based on statistics to summarize and quantify knowledge acquired through prior published research. Meta-analytic methods were initially developed in psychology, medicine and social sciences a few decades ago. In general, meta-analyses are conducted for one of the following four objectives:

- For **Global hypothesis testing**, such as testing for the effect of a certain drug or of a feed additive using the outcomes of many publications that had as an objective the testing of such effect. This was by far the predominant objective of the first meta-analyses published (Mantel and Haenszel, 1959; Glass, 1976). Early on, it was realized that many studies lacked statistical power for statistical testing, so that the aggregation of results from many studies would lead to much greater power (hence lower type II error), more precise point estimation of the magnitude of effects, and narrower confidence intervals of the estimated effects.
- For **Empirical modelling of biological responses**, such as the response of animals to nutritional practices. Because the data extracted from many publications cover a much wider set of experimental conditions than those of each individual study, conclusions and models derived from the whole set have a much greater likelihood of yielding relevant predictions to assist decision-makers. There are numerous examples of such application of meta-analytical methods in recent nutrition publications, such as the quantification of the physiological response of ruminants to types of dietary starch (Offner and Sauvant, 2004), grain processing (Firkins et al., 2001) and rumen definition (Eugene et al., 2004). Others have used meta-analyses to quantify *in situ* starch degradation (Offner et al., 2003), and microbial nitrogen flow in ruminants (Oldick et al., 1999, St-Pierre, 2003).
- For **collective summarizations of measurements that only had a secondary or minor role in prior experiments**. Generally, results are reported with the objective of supporting the hypothesis related to the effect of one or a few experimental factors. For example, ruminal VFA concentrations are reported in studies investigating the effects of dietary starch, or forage types. None of these studies have as an objective the prediction of ruminal VFAs. But the aggregation of measurements from many studies can lead to a better understanding of factors controlling

VFA concentrations, or allow the establishment of new research hypotheses. A meta-analysis of ruminal liquid flow rates allowed the identification of an indirect criterion to saliva production and buffer recycling, which criterion is linked to ruminal conditions (Sauvant and Mertens, 2000).

- **In mechanistic modeling, for parameter estimates and estimates of initial conditions of state variables**. Mechanistic models require parameterization, and meta-analyses offer a mechanism of estimation that makes parameter estimation more precise and more applicable to a broader range of conditions. Meta-analyses can also be used for external model validation, or for a critical comparison of alternate mechanistic models (Offner and Sauvant, 2004).

2.4.1. Types of data and factors in meta-analyses

As in conventional statistical analyses, dependent variables in meta-analyses can be of various types such as binary [0, 1] (*e.g.*, for pregnancy), counts or percentages, categorical-ordinal (good, very good, excellent), and continuous, which is the most frequent type in meta-analyses related to nutrition.

Independent factors (or variables) have either a fixed or random effects on the dependent variables of interest (McCulloch and Searle, 2001). In general, factors related to nutrition (grain types, DMI, etc.) should be considered as fixed effects factors. The study effect can either be considered as random or fixed. If a dataset comprised many individual studies from multiple research centres, the study effect should be considered random because each study is conceptually a random outcome from a large population of studies to which inference is to be made (St-Pierre, 2001). The later indicate that this is especially important if the meta-analysis has for objective the empirical modelling of biological responses, or the collective summarizations of measurements that only had a secondary or minor role in prior experiments. When each experiment can be considered as an outcome each from a different population, the levels of study are in essence arbitrarily chosen by the research community, and the study effect should then be considered fixed. In this case, St-Pierre (2001) suggest that the range of inference for the meta-analysis be limited to the domain of the specific experiments in the dataset.

Glass (1976) defined a meta-analysis as: “The statistical analysis of a large collection of analysis results for the purpose of integrating the findings”. Meta-analyses are generally carried out with

the use of computer and statistical programmes like SAS (DeCoster, 2004). A meta-analysis is a statistical procedure where results from separate studies are incorporated (Crombie and Davies, 2009). The size and quality of each individual study are taken into account, since a weight factor is assigned to each study. According to Crombie and Davies (2009), a proper meta-analysis should involve all studies with similar hypotheses, in attempt to detect possible heterogeneity, while assessing the strength of the main effects.

Meta-analyses are mostly performed on results from quantitative type of experiments where a factor has been studied under several different conditions. The overall impact of the factor is then determined (DeCoster, 2004). Meta-analyses can also be used in primary studies to describe or give background on the research hypotheses, or to explain possible correlations within the primary studies (DeCoster, 2004). Meta-analyses prove to be valuable statistical procedures, on condition that researchers reveal positive as well as negative findings (Dickersin et al., 1987). Since results of meta-analyses are more accurate, trustworthy and maintain a high level of confidence, it may benefit future studies (Sacks et al., 1987).

The intention of researchers performing meta-analyses should be to incorporate all studies, despite its value or accuracy, to reveal the actual results (Glass, 1976; Rosenthal, 1991; Wolf, 1986). Researchers can either include all the results from each and every study, with a weight factor assigned to each study, or execute individual meta-analyses on each study, after which the findings are compared (Rosenthal, 1984; Wolf, 1986; Hunter and Schmidt, 1990).

In studies with a considerable amount of variation, due to effects of animals, feed or environment, meta-analyses are essential in order to detect and verify minor statistical differences (Meissner et al., 2010).

2.4.2. Considerations in meta-analysis

Majority of meta-analyses are based on a series of studies to produce a point estimate of an effect and measures of the precision of that estimate (Stewart and Clarke, 1995). However methods have been developed for meta-analyses to be conducted on data obtained from original trials.

This approach may be considered the “gold standard” in meta-analysis because it offers advantages over analyses using aggregated data, including a greater ability to validate the quality of data, and to conduct appropriate statistical analysis (Simmonds et al., 2005).

2.4.3. Advantages of meta-analysis

According to Cooper, (1997) conceptually, a meta-analysis uses a statistical approach to combine the results from multiple studies. Its advantages can therefore be interpreted as follows:
The results can be generalized to a larger population, the precision and accuracy of the estimates can be improved as more data is used. This in turn may increase the statistical power to detect an effect. Inconsistency of the results across studies can be quantified and analysed. For instance, does inconsistency arise from sampling error, or are study results (partially) influenced by between study heterogeneity. Hypothesis testing can be applied on summary estimates, moderators can be included to explain variation between studies and the presence of publication bias can be investigated.

2.4.4. Reducing bias

DeCoster (2004) stated that the concept of meta-analyses being biased, as it merely incorporates considerable results or outcomes, is untrue. A valuable meta-analysis aims to locate unpublished and minor findings. Bias can easily appear when studies with unfavourable results are excluded from reviews, with researchers generating their own opinions. Meta-analyses may reduce or even eliminate potential bias of experimental information, due to the accurate and methodological nature of the procedure (Crombie and Davies, 2009).

2.4.5. Increased precision

Since the findings of all relevant studies are included in the meta-analysis procedure, the effective sample size is automatically increased. Even the slightest significant effect can be

identified with a higher level of precision, due to a larger number of animals involved in the meta-analysis (Crombie and Davies, 2009).

2.4.6. Transparency

The methods of meta-analyses are generally well stipulated. All decisions and steps during the procedure are recorded, which verifies the validity of the analysis to the readers (Crombie and Davies, (2009).

2.4.7. Disadvantages of meta-analyses

Meta-analyses are accused of: containing one-sided information, since researchers are likely to distribute only beneficial results; the loss of minor details when figures are summarised to determine the general effect, the incorporation of studies with inaccurate or missing information; certain variables being overlooked, since findings from trials with distinct treatments are pooled (Mann, 1990; Pollreisz et al., 1991; Van Donkersgoed, 1992).

2.4.8. Qualitative variation

Although it is frequently stated that meta-analyses fail to account for qualitative variation between studies, DeCoster (2004) explains that the power and effect of these variables are effortlessly retrievable and statistically calculated.

2.4.9. Quality of primary studies

When the quality of information or data to be evaluated in a meta-analysis is low, it will consequently result in a poor meta-analysis. However, since it is possible to statistically determine the quality of studies, inferior studies may be eliminated from the meta-analysis DeCoster, (2004).

2.4.10. Subjectivity

DeCoster (2004) confirms that although meta-analyses are generally perceived as subjective, the mutual subjective outcomes are openly presented and exposed to criticism.

2.4.11. Conducting a meta-analysis

The quality of initial reviews is vital for a valuable meta-analysis. The initial reviews must be accurate and complete and should undergo proper methodological assessment (Bailar, 1997).

2.4.11.1. Methods and quality assessment

According to DeCoster (2004), the method comprises:

- Firstly, identify the hypothesis or topic under investigation.
- Secondly, gather information by selecting individual studies, with related research hypotheses.
- Thirdly, the power and influence of each study have to be statistically calculated
- Fourthly, analyse every possible effects. Lastly, interpret the results by describing the consequences and power of the effects

An established standard should determine whether original studies should be accepted or declined from the meta-analysis (Cook et al., 1995).

2.4.11.2. Heterogeneity and data filtering

A meta-analysis is defined as a statistical analysis which combines the results of several independent clinical trials considered by the analyst to be combinable (Huque, 1988). When heterogeneity is not present in the analysis, a fixed-effect model is used in the statistical procedure. It is then assumed that the difference between studies is only due to chance. In the presence of heterogeneity, a random-effects model is used (Crombie and Davies, 2009). Selected studies should coincide with the relevant aims of the meta-analysis and should be assessed to

ensure that no inaccuracies are present. The information should also be validated in the database and extreme values should be handled carefully (Sauvant et al., 2008).

The purpose of meta-analyses is to produce new information from existing records (Sauvant et al., 2008). In contrast to individual studies, meta-analyses have substantially more supporting data, allowing this statistical procedure to generate significant findings, which are exceptionally accurate and reliable (Crombie and Davies, 2009). In addition, meta-analysis gives an academic interpretation of findings and describes the consistency and suggests possible improvement or further development in future analyses, by yielding new evidence (DeCoster, 2004). The primary reasons for utilising a meta-analysis in this study are the large dataset, studies have similar hypotheses, positive and negative results are included and because of considerable variation in data due to the effects of environment, feed and animals.

CHAPTER 3

3. MATERIALS AND METHODS

3.1. Meta-analysis of effects of Yeast culture and Monensin

A literature search and screening process yielded a total of 9 papers for yeast and 10 for monensin were identified with sufficient data and quality to production outcomes. The available trials provided approximately 840 (400 for yeast; 440 for monensin) cows with sufficient data for analysis. This provided good statistical power to examine the effects of both yeast and monensin. All trials included were randomized designs using total mixed ration and Holstein cows. Data from each trial contained in the papers was extracted to a database including the number of animals, mean, and standard error for each of the yeast, monensin and control groups.

Meta-analysis was conducted based on means and standard errors using Practical Meta-Analysis Effect Size Calculator (Lipsey and Wilson, 2001) for yeast and monensin effects. Analysis was done on milk yield, dry matter intake, milk fat and protein yield, energy corrected milk, body weight, milk urea nitrogen, feed efficiency. Urinary nitrogen excretion was estimated as described by Kauffman and St-Pierre, (2001):

Urinary N, g/d = 0.0259 x milk urea N (mg/dl) x BW (kg) (1); Urinary N, g/d = 15.1 x MUN + 27.8 (2)

The standardized mean-difference effect size, confidence interval and P-value were calculated. The percentage change was calculated from the mean value of each control and treatment.
The standardized mean-difference effect size (d) was calculated as:

$$d = \frac{\overline{X}_{G1} - \overline{X}_{G2}}{s_{pooled}}$$

$$s_{pooled} = \sqrt{\frac{s_1^2(n_1 - 1) + s_2^2(n_2 - 1)}{n_1 + n_2 - 2}}$$

Where

$\overline{X}_{G1}$: The mean of the control group

$\overline{X}_{G2}$: The mean of the yeast or monensin group

n_1 : Control group population

n_2 : Yeast or monensin group population

S_{pooled} = Pooled standard error

s_1^2 : Square error of the control group

s_2^2 : Square error of the yeast or monensin group

The confidence interval (95%) was calculated as:

$$Lower = d - 1.96(S_{pooled})$$

$$Upper = d + 1.96(S_{pooled})$$

Differences were declared significant at $P < 0.05$ and a tendency toward significance was discussed at $0.5 < P < 0.10$.

3.2 Effects of Live yeast and monensin on gas production and rumen fermentation *in vitro*

3.2.1. Basal diet, treatments and design

Three additives (LY, M and LY+M) were tested individually in three (3) different runs. Using high or low concentrate: forage (C:F) ratio diet. Treatments were arranged in a complete randomized design, 2 x 2 factorial design using two treatments, control (C) vs. additive, with each treatment using two concentrate to forage (C:F) ratio (60:40 and 40:60). The basal diet was a total mixed ration formulated to fulfil the minimum nutrient requirement of an early lactating 680 kg Holstein cow producing 40 kg of milk with 4% fat and 3.5% protein (NRC, 2001). The ingredient and chemical composition of the diet are in Table 1. Live yeast treatment was 0.25 g/kg of feed, M was 33 mg/ kg of feed and LY+M was 0.25 g LY+33 mg M per kg feed.

Table 1: Ingredient and chemical composition of the diets

Ingredients (%)	C:F ratio	
	60:40	**40:60**
Concentrate mixture[a]	45.8	26.0
Whole cotton seed	7.2	7.0
Molasses meal	7.2	7.2
Maize silage	-	3.1
Alfalfa hay	23.9	40.8
Eragrostis hay	13.9	16.0
Vitamin/mineral premix[bc]	0.1	0.1
Chemical composition (% DM)		
$NE_{L,}$ MJ /kg DM[d]	5.75	5.69
Crude protein (CP)	15.50	15.60
Rumen un-degraded protein (RUP)	5.20	5.00
Acid detergent protein (ADF)	16.00	16.20
Neutral detergent fibre (NDF)	31.70	36.90
Fat (F)	4.40	4.31
Calcium (Ca)	0.79	0.77
Phosphorus (P)	0.39	5.69

[a]DM of the maize based concentrate mixture contained: 19 % CP, 7.5 MJ/kg NE_L, and 5% Fat.

[b]Contains per kg of premix: 7,000k Iu of Vitamin A; 1,500k Iu of Vitamin D_3; 1300 mg of Vitamin B_1; 4000 mg of Vit B_{12}; 15,000 mg of Vit E; 130,000 mg of niacin; 1000 mg of Co; 3000 mg of I; 375 mg of Se; 100,000 mg of Mn; 20,000 mg of Cu; 100,000 mg of Zn; 350,000 mg of S; 60,000 mg of Fe

There were four mineral/vitamin premixes, with the treatments being created by addition of virginiamycin or monensin, or both, at a level to provide 20 ppm of virginiamycin or 15 ppm of monensin in the DM of the TMR

[d]Calculated using the NRC (2001) recommendations.

3.2.2 Preparation of *in vitro* medium and reducing solution

The reduced buffer solution for the *in vitro* techniques (*in vitro* GP system and ANKOM® technique) was based upon the *in vitro* rumen digestibility buffer solution. Medium was prepared as described by Goering and Van Soest (1970). The medium and the reduced buffer solution are presented in Tables 2 and 3 respectively.

Table 2: The reduced buffer solution used in the *in vitro* digestion

Composite	volume
Distilled water (ml)	500
Tryptose (g)	2.5
Resazurin 0.1% w/v (ml)	1.25
Macro mineral (ml)	250
Micro mineral (ml)	0.125
Buffer solution (ml)	250
Reducing solution (ml)	50

The medium was kept in a water bath at 39.0 ºC and mixed with the reducing solution while being flushed with CO_2 in order to enhance the mixture of the solution and to induce anaerobic condition. The media was then sealed and left in the water bath at 39.0 ºC to reduce anaerobic condition.

Table 3: Composition of the *in vitro* buffer solution

Macro mineral	**Reagents**	**1l volume**
	Distilled water	1000
	Na_2HPO_4 anhydrous (g)	5.7
	KH_2PO_4 anydrous (g)	6.2
	$MgSO_4.7H_2O$ (g)	0.59
	NaCl (g)	2.22
Micro mineral	**Reagents**	**100ml volume**
	Distilled water (ml)	100
	$CaCl_2.2H_2O$ (g)	13.2
	$MnCl_2.4H_2O$ (g)	10
	$CoCl_2.6H_2O$ (g)	1
	$FeCl_3.6H_2O$ (g)	8
Buffer solution	**Reagents**	**1l volume**
	Distilled water (ml)	1000
	NH_4HCO_3 (g)	4
	$NaHCO_3$ (g)	35
Reducing solution	**Reagents**	**100ml volume**
	Distilled water (ml)	100
	Cysteine Hydrochloric acid (g)	0.625
	KOH pellets (g)	10
	Na Sulphide non hydrate (g)	0.625

The maintenance of temperature at 39.5 ºC as well as the reduced state of the buffer solution would respectively limit temperature and aerobic shock to rumen microbes when rumen fluid is mixed with the buffer solution (Mertens and Weimer, 1998).

As recommended by Tilley and Terry (1963), a ratio of 40:10 ml of reduced media to rumen liquor is adequate to maintain a pH ambiance within the usual limits for digestion to ensure that the final acid concentration does not exceed that found in the animal.

3.2.3. Collection and preparation of rumen fluid

Rumen liquor was collected and rumen content was squeezed through two layers of cheese cloth into pre-warmed flasks and a small amount of inoculum was added. The flasks were completely filled before being capped to keep the anaerobic milieu while they were transported to the laboratory. The rumen fluid with inoculum was blended in a pre-warmed industrial blender (Waring Commercial® Heavy Duty Blender, Waring® Corporation, New Hartford, CT, USA), at a low speed for 10 seconds to free bacteria that may be attached to solids (Goering and Van Soest, 1970). The rumen fluid was then filtered through two layers of cheese cloth into beakers and maintained at 39 ºC in the water bath while being flushed with carbon dioxide (CO_2) to sustain anaerobic condition.

3.2.4. *In vitro* gas production system

In vitro GP system and mixed culture fermentation were conducted with the reduced buffer solution and rumen liquor collected from a cannulated lactating Holstein cow. Treatments consisted of basal diet with no additive, basal diet with monensin, basal diet with yeast culture and basal diet plus monensin + yeast. The basal diet was a balanced total mixed ration balanced for an early lactating Holstein cow. The yeast preparation (Levucell© SC, Lallemand Animal nutrition, USA) is a commercial direct-fed microbial product containing *Saccharomyces cerevisiae* at a ratio of 3.3 x 10^9 cfu/g. Monensin is a carboxylic polyther ionophore produced by naturally occurring strains of *Streptomyces cinnamonensis* (Haney and Hoelm, 1967).

Glass vials of 250 ml of volume were used in the *in vitro* GP technique. Feed samples of 0.5±0.005 g were weighed into bottles containing a magnetic stirrer each. These bottles were then flushed with CO_2 after adding 40 ml of reduced buffer solution to each bottle. The bottles were closed and placed in a water bath at 39.5ºC until the medium was reduced (clear), after which the bottles were re-opened and 10 ml of rumen fluid added while flushing with CO_2. The bottles were then closed tightly with rubber stoppers, crimp sealed and connected to a pressure transducer system in the incubator at 39ºC. Three bottles with only rumen liquor and reduced buffer solution were also included in each test as blanks for correction of gas produced. All

bottles were zeroed in terms of gas produced by opening their valves before the beginning of the incubation. Forty eight hours were used as period of incubation and gas pressure was recorded automatically using a pressure transducer system (Eagle Technology Ltd.) based on the methods by Pell and Schofied, (1993).

Gas measurements recorded at each interval were in terms of pressure (psi units). The psi pressure was later converted into volume as millilitres of gas produced using a calibration curve and the subsequent regression equation of pressure against volume for each bottle.

The pressure of each bottle was then measured and the net pressure for each bottle was estimated. This was done by subtracting the average pressure measured for two bottles where gas was not added from all the other pressures measured as correction for the gas produced from the added inoculum and buffer solution. The volume fraction of each bottle was then plotted against the net pressure measured within each bottle. The calibration curve and the regression equation, as described by Goosen (2005), showed a good correlation (R^2=0.9904) between the net pressure measured and the volume fraction of the bottle. Thus, the regression equation of y=0.0977x was used as standard regression equation to convert the pressure readings measured experimentally to a volume fraction. This calculated volume fraction would then be multiplied by the head space or known gas phase volume of each bottle to give the volume of gas produced in millilitres as follows:

- Pressure (ml) at time t = 1000 x (0.0977 x Net pressure x head space) / OM with:
- Net pressure (psi units) at time t = Psi produced from substrate bottle – Psi from blank bottle;
- Head space of bottle (ml) = volume vial – 52.5;

3.2.5. *In vitro* ruminal fermentation

Feed samples of 0.5±0.005 g were weighed into bottles containing a magnetic stirrer each. These bottles were then flushed with CO_2 after adding 40 ml of reduced buffer solution to each bottle. The bottles were closed and placed in a water bath at 39.5ºC until the medium was reduced (clear), after which the bottles were re-opened and 10 ml of rumen fluid added while flushing with CO_2. The bottles were then closed tightly with rubber stoppers, crimp sealed to contain the

gas pressure and placed in a 39°C water bath and periodically mixed. After 24 of incubation, bottles were sampled for VFA and ammonia nitrogen determination.

3.2.5.1. Volatile fatty acid's determination

Volatile fatty acids were analyzed by gas chromatography. The preparation of samples for determination of VFAs by gas chromatography method was based on the procedure of Manni and Caron, (1995). The samples were acidified to pH 2 using 65% of nitric acid. A 1 mL portions were shaken along with a 1 mL of diethyl ether for approximately 10 min, and the ether phases were quantitatively transferred to 4-mL flasks, where a small amount of anhydrous sodium sulphate was added. The 500 μL portions of ether phases were transferred into new 4-mLflasks and 150 μL of diazomethane was added.

A series of VFA standards for the calibration curves were prepared in the same manner as described above. Calibration curves were obtained using five aqueous solutions of acids: acetic, propionic, butyric, in the concentration range of 5 to 1000 mg/ml.

Gas chromatography analyses were performed on a GC 8000 TOP (CE Instruments) gas chromatograph equipped with a flame ionization detector and a DB-23 capillary column (30 m, 0.25 mm I.D., 0.25 μm film thickness, Alltech, Poland). The injector and detector temperatures were both 170°C. The carrier gas was argon. The analyses were performed using a temperature programme: 5 min at 30°C and a linear gradient from 30°C to 130°C at 10°C min-1. In each case a 2 μL of sample was injected (a flow splitting 1:10).

3.2.5.2 Ammonia nitrogen determination

Ammonia was determined by serie-automated colorimetry (O'Dell, 1993). The sample was buffered at a pH of 9.5 with a borate buffer in order to decrease hydrolysis of cyanates and organic nitrogen compounds, and was distilled into a solution of boric acid. Alkaline phenol and hypochlorite react with ammonia to form indophenol blue that is proportional to the ammonia concentration. The blue color formed was intensified with sodium nitroprusside and measured colorimetrically.

3.3. Statistical analysis

Data was analysed as a complete randomised design with a 2 × 2 factorial treatment arrangement by using the GLM procedures (SAS, 2009). The model included the fixed effects of treatments (additive, diet C:F, and the additive × diet C:F interaction) as main effects. The mean squares of the dietary effects were divided into three orthogonal contrasts considering the concentrate to forage ratios (60:40 vs. 40:60), the additive (monensin, live yeast + monensin) treatment (control vs. treated), and the interaction between these factors (60:40 control + 40:60 treated vs. 60:40 treated + 40:60 control). Differences were declared significant at $P \leq 0.05$ and a tendency toward significance was discussed at $0.5 < P \leq 0.10$.

CHAPTER 4
RESULTS

4.1. Meta-analysis of effects of yeast and monensin on production parameters

The results on effect size of yeast and monensin on production parameters are presented in Table 4 and 5 respectively.

Table 4: Summary of effect size estimates of yeast on production and calculated nitrogen excretion in lactating dairy cows derived from meta-analysis

Parameters	Weighted mean difference (Treat-control)	% change	Confidence interval (95%)		Effect size P-value
Dry matter intake, kg/d	0.13	3.5	-0.27	0.61	0.07
Milk yield, kg/d	-0.35	-10.8	-0.79	0.09	0.15
Milk fat yield, kg/d	-0.58	-17.6	-1.13	-0.23	0.04
Milk crude protein yield, kg/d	-0.21	-5.3	-0.64	0.23	0.06
Body weight, kg	-0.32	-2.0	-0.76	0.12	0.05
Milk urea nitrogen, mg/dl	-0.11	-1.8	-0.54	0.33	0.08
Energy corrected milk, kg/d	-0.50	-12.8	-0.94	-0.06	0.04
Feed efficiency	-0.56	-15.5	-1.09	-0.20	0.04
Urinary nitrogen 2, g/d	-0.11	-3.9	-0.54	0.33	0.11
Urinary nitrogen 1, g/d	-0.20	-1.6	-0.64	0.23	0.04

Over all the trials analyzed, addition of yeast tended ($P<0.10$) to increase DMI with no effect ($P>0.05$) on milk yield. Milk fat and crude protein yield were increased ($P<0.05$) and tended ($P<010$) to be decreased respectively. Cow's BW, MUN and urinary nitrogen were not affected, but ECM and FE were decreased. Weighted means of the difference for milk fat yield, ECM and FE suggested a 0.6 kg/day, 0.5 kg/day and 0.6 decrease respectively.

Table 5: Summary of effect size estimates of monensin on production and calculated nitrogen excretion in lactating dairy cows derived from meta-analysis

Parameters	Weighted mean difference (Treat-control)	% change	Confidence interval (95%)		Effect size P-value
Dry matter intake, kg/d	-0.51	-5.5	-0.85	0.03	0.04
Milk yield, kg/d	0.56	21.3	-0.08	0.80	0.03
Fat yield, kg/d	-0.57	16.2	0.13	1.02	0.04
Crude protein yield, kg/d	0.18	1.2	-0.25	0.62	0.09
Body weight, kg	0.50	17.0	0.25	1.15	0.04
Milk urea nitrogen, mg/dl	0.59	13.8	0.14	1.03	0.04
Energy corrected milk, kg/d	0.49	24.2	0.05	0.94	0.05
Feed efficiency	0.34	11.2	-0.29	0.58	0.04
Urinary nitrogen 2, g/d	0.39	20.0	-0.05	0.83	0.05
Urinary nitrogen 1, g/d	0.56	27.7	0.12	1.01	0.05

Addition of monensin decreased and increased DMI but milk production respectively ($P<0.05$). Milk fat and protein yield were decreased and tended to be increased respectively ($P<0.05$). Body weight, MUN and FE were slightly increased ($P<0.05$), but ECM and urinary nitrogen were not affected ($P=0.05$). The effect size estimates for monensin on milk fat percent, milk fat yield, milk protein percent and milk protein yield were heterogenous and random effects models were utilized for these variables. Weighted means of the difference for milk production and dry matter intake suggested a 0.71 kg/day increase and a 0.56 kg/day decrease respectively.

4.2. Effects of live yeast and monensin on cumulative gas production of two diets differing in concentrate to forage ratios

Figure 2 shows the average cumulative *in vitro* gas production as affected by LY, M and LY+M in high (60:40) and low (40:60) C:F ratio diets. Overall, gas (ml) produced by 40:60 diets were

below the levels produced by 60:40 diets, but no differences (P<0.05) were observed between treatment within diets (40:60 and 60:40).

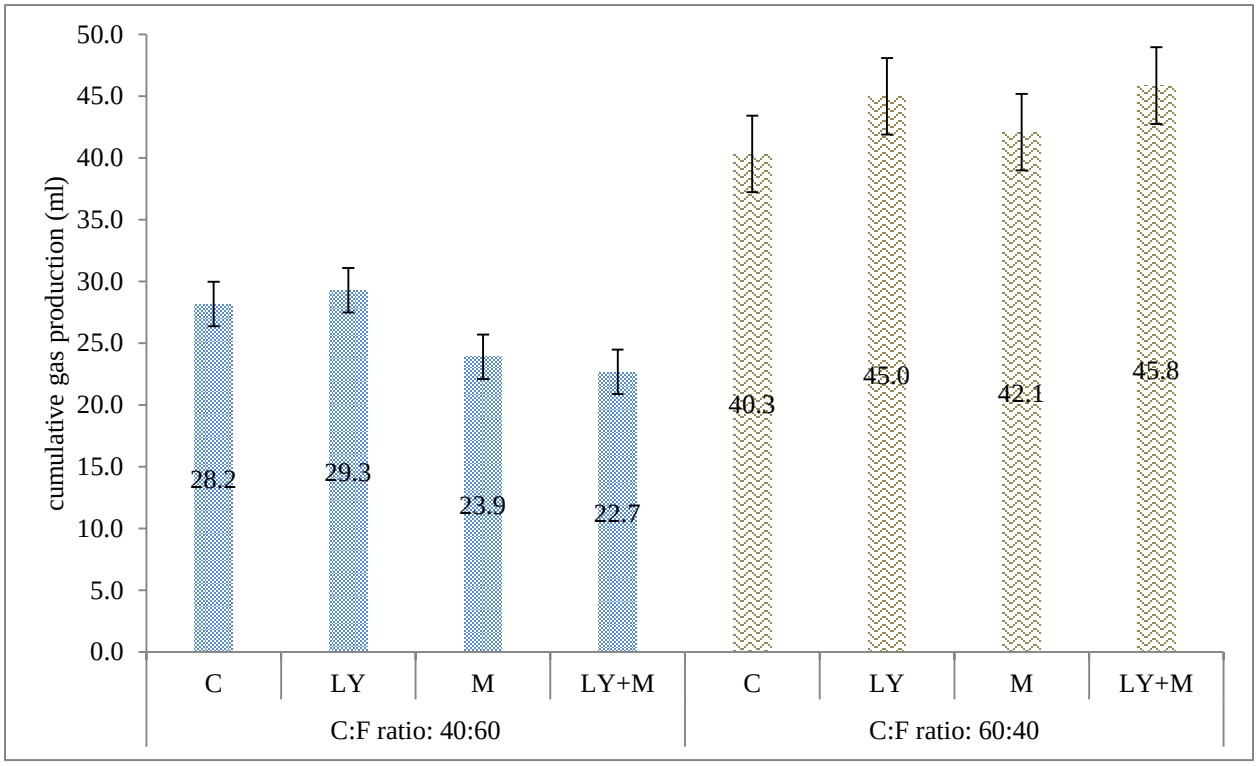

Figure 2: The average cumulative gas produced by high (60:40) and low (40:60) concentrate diets supplemented or not (C) with live yeast (LY), monensin (M) or their combination (LY+M)

When compared to control, in 40:60 diets, LY produced 4.0 % more gas (Figures 3 and 4), while M and the combination treatments (LY+M) decreased gas by 14.2 and 19.5%. In 60:40 diets (Figure 4), gas production was increased by addition with LY, M and LY+M by 11.6, 4.0 and 13.7 %, respectively compared to the control. An illustration of the cumulative gas production overtime is shown in Figures 3 and 4.

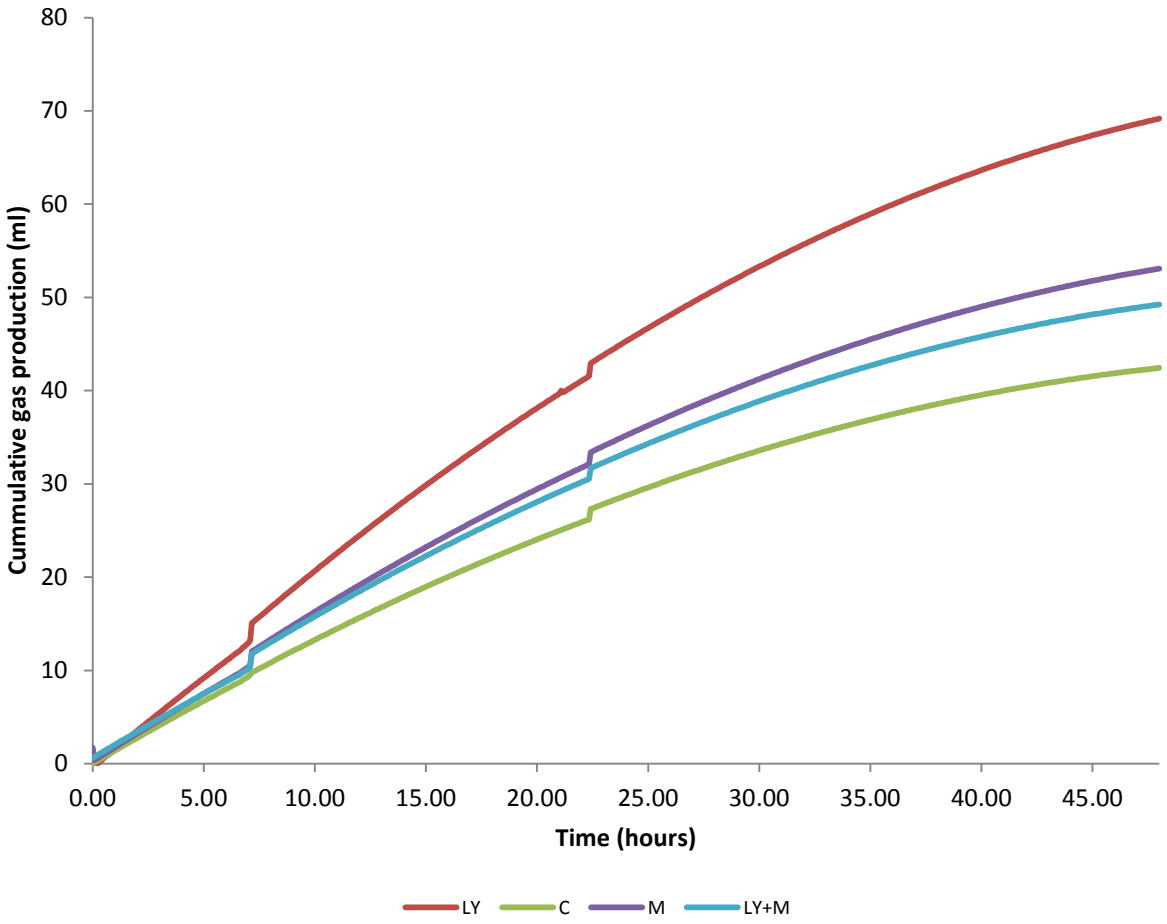

Figure 3: Cumulative GP (ml) profiles of low concentrate: forage ratio (40:60) diet as affected by supplementation with live yeast (LY), monensin (M), their combination (LY+M) or control (C). HC: High concentrate (60:40); LC: Low concentrates (40:60) diets

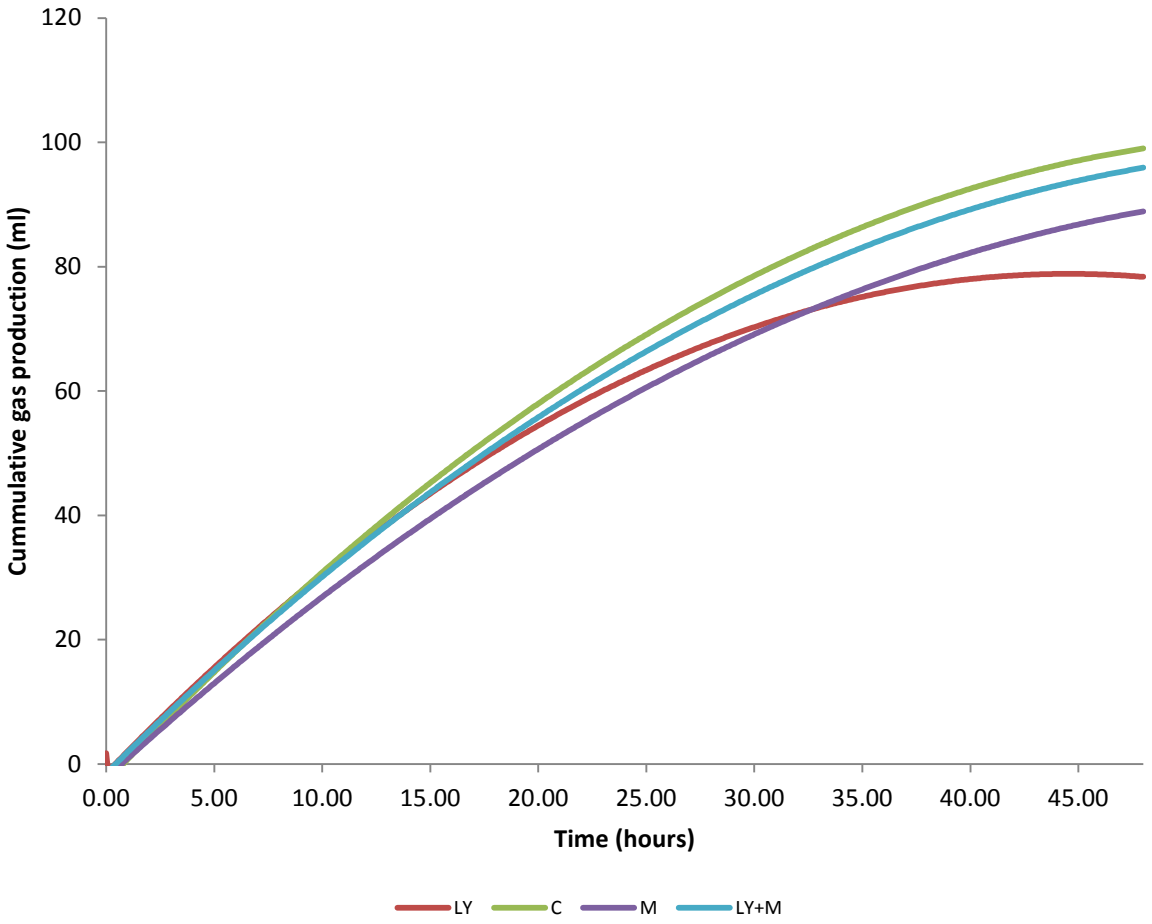

Figure 4: Cumulative GP (ml) profiles of by diet as affected by high concentrate: forage ratio (60:40) diet supplementation with live yeast (LY), monensin (M), their combination (LY+M) or control (C). HC: High concentrate (60:40); LC: Low concentrates (40:60) diets

In both low and high concentrate diets, cumulative gas increased with time in all treatments from 0 to 45 hours (h). In 40:60 diets the control was surpassed by all other treatments with LY being above all treatments followed by M and LY+M. In 60:40 diets (Figure 3) C suppressed the other treatments followed by LY+M and M. Results on contrast effects of LY, M and LY+M are presented in Tables 6; 7 and 8 respectively.

Table 6: Effects of live yeast (LY) and ratio of concentrate to forage (C:F) on gas production

	C:F				SEM	Contrast		
	40:60		60:40					
	C	LY	C	LY		LY	F:C	LY vs. C:F
1.5 h	1.06	1.04	1.34	1.14	0.17	0.11	<0.01	0.09
12 h	21.7	23.9	31.9	32.4	2.14	0.19	0.06	0.87
24 h	34.9	32.1	49.9	52.7	4.10	0.06	<0.01	0.04
48 h	55.0	60.1	78.1	93.7	6.50	0.04	<0.01	0.01

C:F= Concentrate to forage ratio, C= Control, LY= Live yeast, M= Monensin, LY+M= Live yeast + Monensin; SEM= Standard error of mean

Addition of LY affect gas produced only at 48 h decreased and increased gas in 40:60 and 60:40 respectively. The C:F ratio affected gas at all incubation times, showing more gas in 60:40 than 40:60 diet. Addition of LY and F:C ratio interacted at 24 and 48 h, with LY increasing gas in 60:40 diet.

Gas production was affected by monensin at 1.5 and 48 h only (Table 7). Ratio of C:F affected gas produced at all incubation times, with only a tendency ($P<0.06$) at 12 h, showing higher gas produced in 60:40 than 40:60 diets. The interaction between M and C:F ratio indicated that monensin increased and decreased gas in 40:60 and 60:40 diets, respectively at 1.5 h. As for LY, addition of M also affected gas production at 48 h of incubation, increasing and decreasing it in 40:60 and 60:40 diets, respectively.

Table 7: Effects of monensin (M) and ratio of concentrate to forage (C:F) on gas production

	C:F				SEM	Contrast		
	40:60		60:40					
	C	M	C	M		M	C:F	M vs. C:F
1.5 h	1.06	1.45	1.34	0.12	0.16	<0.01	<0.01	<0.01
12 h	21.7	17.0	31.9	28.2	2.30	0.19	0.06	0.87
24 h	34.9	27.1	49.9	46.1	5.36	0.14	0.01	0.67
48 h	55.0	50.0	78.1	93.9	8.14	0.04	0.02	0.03

C:F= Concentrate to forage ratio, C= Control, LY= Live yeast, M= Monensin, LY+M= Live yeast + Monensin; SEM= Standard error of mean

Table 8: Effects of live yeast and monensin (LY+M) and ratio of concentrate to forage (C:F) on gas production

	C:F				SEM	Contrast		
	40:60		60:40					
	C	LY+M	C	LY+M		LY+M	C:F	LY+M vs. C:F
1.5 h	1.07	1.68	1.34	1.07	0.08	0.09	0.11	<0.01
12 h	21.7	16.5	31.9	31.6	2.58	0.33	<0.01	0.39
24 h	34.9	25.9	49.9	50.8	3.65	0.04	<0.01	0.03
48 h	55.0	46.6	78.1	99.9	5.77	0.70	<0.01	0.05

C:F= Concentrate to forage ratio, C= Control, LY= Live yeast, M= Monensin, LY+M= Live yeast + Monensin; SEM= Standard error of mean

The combination treatment (LY+M) affected gas at 1.5, 24 and 48 h, but not at 12 h. Gas production was not affect by LY+M at 1.5 h but was so at 12, 24 and 48 h, showing higher gas produced in 60:40 than 40:60 diets. The interaction between M and C:F ratio indicated that monensin increased and decreased gas in 40:60 and 60:40 diets, respectively at 1.5 h, but the opposite was observed at 24 and 48 h.

4.3. Effects of live yeast and monensin on ammonia nitrogen and volatile fatty acids of two diets differing in concentrate to forage ratios

Effects of live yeast, monensin and their combination in low and high ratio of concentrate to forage (C:F) on volatile fatty acids and ammonia nitrogen are presented in Tables 9, 10 and 11.
Addition of LY did not affect ammonia, total VFA and individual VFA proportion (Table 9). However, LY tended ($P<0.10$) to decrease acetate proportion. The proportion of acetate was significantly affected by the C:F ratio. There was not interaction effects of LY and C:F ratio on all the fermentation parameters.

Addition of LY had no effect on acetate, but acetate concentrate tended to be decreased in low concentrate diet (Table 10). The lack of effect of yeast on ruminal propionate observed in this study does not agree with other study (Harrison et al., 1988, Newbold et al., 1990, Plata et al., 1994) that reported increased proportion of propionate.

Butyrate was not affected by LY in the present study. Henderson et al., (1981) suggested that the differences in the *Butyrivibrio* rumen bacteria communities between studies may also contribute to the increased variation in the change in butyrate concentration in the rumen.

The main effects of the forage to concentrate ratios were also as expected. However, the addition of LY tended to increase and decrease total VFA's in high and low concentrate diet, respectively. In addition, the decreased of acetate was more with the low forage diet than with the high forage diet (interaction of monensin and forage to concentrate ratio, $P < 0.01$).

Table 9: Effects of live yeast (LY) and ratio of concentrate to forage (C:F) on volatile fatty acids and ammonia nitrogen

	C:F				SEM	Contrast		
	60:40		40:60					
	C	LY	C	LY		LY	C:F	LY vs. C:F
Ammonia nitrogen	6.35	6.98	5.88	6.34	0.22	0.22	0.21	0.85
Total VFA	133.6	136.5	123.7	113.7	4.32	0.68	0.06	0.45
Acetate acid	64.8	64.22	68.00	67.80	0.37	0.08	<0.01	0.31
Propionate acid	16.2	16.2	15.3	15.7	0.70	0.94	0.68	0.94
Butyrate acid	12.9	14.1	14.6	15.6	0.52	0.28	0.12	0.92
Valerate	1.68	1.68	1.70	1.71	0.10	0.87	0.97	0.97
Iso butyrate	1.67	1.67	1.68	1.68	0.10	0.99	0.94	0.99
Iso valeric acid	2.75	2.13	1.69	1.69	0.11	0.22	0.09	0.11
A:P ratio	4.1	4.1	4.5	4.5	0.17	0.92	0.30	0.98

C:F= concentrate to forage ratio, C= Control, LY= live yeast, M= Monensin, LY+M= live yeast + Monensin; SEM= Standard error of mean, A:P ratio: Acetate to propionate ratio.

Table 10: Effects of monensin (M) and ratio of concentrate to forage (C:F) on ruminal fluid composition

	C:F				SEM	Contrast		
	60:40		40:60					
	C	M	C	M		M	C:F	M vs. C:F
Ammonia nitrogen	6.35	5.98	5.88	5.41	0.26	0.14	<0.01	0.01
Total VFA	133.6	145.5	123.7	129.8	4.03	0.26	0.12	0.72
Acetate acid	64.8	63.0	68.0	67.1	0.41	<0.01	<0.01	0.03
Propionate acid	16.2	18.6	15.3	17.6	0.54	0.04	0.08	0.86
Butyrate acid	12.9	12.5	14.6	14.7	0.51	0.87	0.06	0.77
Valerate	1.68	1.72	1.68	1.75	0.09	0.78	0.94	0.94
Iso butyrate	1.67	1.61	1.68	1.45	0.09	0.46	0.72	0.66
Iso valeric acid	2.75	2.59	2.77	3.14	0.10	0.61	0.16	0.21
A:P ratio	4.1	3.4	4.5	3.9	0.17	0.01	0.11	0.92

C= Control, LY= live yeast, M= Monensin, LY+M= live yeast + Monensin; SEM= Standard error of mean, A: P ratio: Acetate to propionate ratio.

Ammonia nitrogen and Total VFA were not affected by monensin (P = 0.14) in Table 10. Ruminal propionate was increased with Addition of M. Because of M, propionate concentration increased, and acetate and butyrate percentages decreased, resulting in lower ratios of acetic acid to propionic acid. No effects of M were observed on concentrations of ammonia N and total VFA, but ammonia N concentration was decreased by addition of M in low concentrate diet (interaction of monensin and forage to concentrate ratio, $P = 0.01$). As for LY, there was a main effect of the forage to concentrate ratios. The addition of monensin increased the percentage of propionate, and decreased that of acetate, more with the low forage diet than with the high forage diet (interaction of monensin and forage to concentrate ratio, $P = 0.03$).

Table 11: Effects of live yeast and monensin (LY+M) and ratio of concentrate to forage (C:F) on ruminal fluid composition

	C:F				SEM	Contrast		
	60:40		40:60					
	C	LY+M	C	LY+M		LY+M	C:F	LY+M vs. C:F
Ammonia nitrogen	6.35	6.40	5.88	6.34	0.14	0.91	0.06	0.77
Total VFA	133.6	123.56	123.7	113.74	4.23	0.06	0.06	0.53
Acetate acid	64.8	63.2	68.0	67.80	0.54	0.79	<0.01	<0.01
Propionate acid	16.2	18.11	15.3	15.71	0.57	0.16	0.06	0.81
Butyrate acid	12.9	12.59	14.6	15.64	0.47	0.51	0.15	0.74
Valerate	1.68	1.60	1.68	1.81	0.09	0.83	0.59	0.51
Iso butyrate	1.67	1.65	1.68	1.71	0.09	0.73	0.87	0.80
Iso valeric acid	2.75	2.85	2.77	1.69	0.11	0.03	0.07	0.10
A:P ratio	4.1	3.55	4.5	2.86	0.14	0.02	0.24	0.75

C= Control, LY= live yeast, M= Monensin, LY+M= live yeast + Monensin; SEM= Standard error of mean, A: P ratio: Acetate to propionate ratio.

Addition of LY+M (Table 11) tended to decrease total VFA's. Iso-valeric concentration was increased and decreased in high and low C:F diets, respectively with addition of LY+M. The acetate to propionate ratio was also increased wit LY+M. There was also tendency of increase and decrease in ammonia nitrogen respectively in low concentrate diet. The acetate concentration was increased in low concentrate diet.

CHAPTER 5
DISCUSSION

5.1. Meta-analysis of effects of yeast and monensin

Meta-analysis of the selected studies did not show improvement of cow's performance with yeast cultures. Dry matter intake tended to be increased with no change in milk yield, which negatively affected milk fat, energy average milk (ECM) and feed efficiency (FE). Based on Cohen (1988), these decreasing effects were medium (0.5: effect size). The positive results were observed on estimated urea nitrogen (UN), which was decreased also with a medium effect. This observation agrees with previous report which indicated that yeast decreases the rate of degradation of peptides, which may reduce ammonia wastage and may increase the amount of rumen undergraded protein (RUP) available to the cow (Chaucheyras-Durand et al., 2005). Continuous culture research done by Moya et al., (2007), on the addition of LY to the diet decreased ammonia, increased bacterial N production and increased the efficiency of microbial protein synthesis. This is explained by the negative effect on *Streptococcus bovis* and protease activities.

The decreasing and increasing effects of monensin on DMI and milk yield, respectively, is consistent with literature (Phipps et al., 2000; Heuer et al., 2001) and this is confirmed by the tendency of increased ECM and increased FE. The decrease in milk fat % is also reported as a consequence of feeding monensin (Duffield et al., 2003). Lactating cows usually lose condition in early lactation. The improved BCS observed with monensin was also reported (Wagner et al., 1999) and can be explained by improved energy status, due to more propionic acid produced in the rumen, minimising use of body reserves.

While meta-analysis of monensin confirmed previous report of monensin effects, the analysis of yeast did not. Although this can be caused be selected studies, it also highlight the importance of the meta-analysis as a useful tool that can be employed to both summarize effects across studies and to investigate factors explaining potential heterogeneity of response.

5.2. Effect of live yeast and monensin on gas production in low and high concentrate diets

The current experiment was designed to test the effect of LY and/or M on *in vitro* ruminal fermentation of low and high C:F ratios. The gas production techniques were used to estimate apparent digestibility by evaluating cumulative gas produced as affected by treatments. *In vitro* methods provide an alternative (Paya et al., 2007) ways for evaluating feed's value and indicate the production of short chain of fatty acids, which increase leads to increased gas produced and results in high digestibility and energetic value (Maheri-Sis et al., 2011).

Addition of LY did not affect gas production during the first 24 h of incubation, which is not in agreement with Lila et al., (2004) who reported an increase in gas when LY was added to high C:F diets. Effects of LY in the present study were observed late at 48 h of incubation. When testing effects of LY on high C:F diet, the results of Opsi et al., (2012) obtained from144 h incubations showed differences in the cumulative gas production only at 24 h of incubation. The later indicated that LY would not induce prompt changes in the ruminal microbial population, suggesting enough time for adaptation. Williams et al., (1991) suggested that ruminal micro-organisms could be stimulated by yeasts at initial stages of fermentation, but these effects would become negligible. Thus, short-term shifts in fermentation pattern could not be expected, which may explain the late increasing effects in the present study.

The significant effect of Monensin and the interaction between M and C:F ratios are unclear since at 12 h there was no effect. Addition of M in this study was effective in increasing gas produced in 60:40 diet, which suggest that the C:F ratio need to be considered if feed digestibility is to be evaluated. These results agree with Anassori et al., (2011) who reported that M addition reduced gas production after 96 h of incubation in low C:F ratio (30:70) of sheep diet.

High concentrate diet has shown to have high impact on gas production. Every time LY, M or LY+M were added to 40:60 diet, cumulative gas produced was reduced or unchanged, but when added to 60:40 diet, it was generally increased. Forage and concentrate levels in diets are responsible for the different responses in dairy cattles (Dewhurst et al., 2001). These interactions

are due to associative effects of dietary components, which affect dietary component's digestibility (Dixon and Stocdale, 1999).

5.3. Effects of live yeast and monensin on ammonia nitrogen and volatiles fatty acid

5.3.1. Effects of live yeast

During fermentation, considerable fraction of the protein consumed by ruminants is fermented to ammonia and volatile fatty acids by ruminal microorganisms (Nolan, 1975). Similar to the present study, Inal et al., (2010) reported no effect of live yeast on rumen ammonia. These finding agree with most previous studies (Mwenya et al., 2005; Newbold et al., 1995; Palkova et al., 1997; Zikanova et al., 2002: Tripathi et al., 2008), which reported that microbial cultures based on *Saccharomyces cerevisiae* had no effect on ruminal ammonia-N. Other *in vivo* studies also reported no effects of yeast on ruminal ammonia nitrogen in sheep (Newbold et al., 1995) and lactating cows (Erasmus et al., 1992). However, some studies reported a decrease (Lascano et al., 2009) or increase (Blauwiekel et al., 1995; Pinos-Rodriguez et al., 2008) in presence of yeast products. Decreased ammonia concentration was also observed by Alshaikh et al., (2002) when yeast culture was supplemented to the high concentrate diet, which can be attributed to more ammonia-N incorporated into ruminal microbial proteins (Carro et al., 1992). Live yeast has negative effect on *Streptococcus bovis)* and proteinase activities (Chaucheyras-Durand et al., 2005), and may decrease the rate of degradation of peptides, which should reduce ammonia wastage and increase the amount of rumen undegraded protein available to the cow. Ammonia production in the presence of yeast is reported to be highly dependent on the availability of amino acids (Palkova et al., 1997; Zikanova et al., 2002) and could also be associated with other characteristics of the diet.

Although molar proportion of acetate tended to decreased with live yeast supplementation, the rest of VFA's and concentration of total VFA were not affected. Other researchers (Arcos-Garcia et al., 2000; Lascana and Heinrichs, 2009) reported increases in total VFA, while other (Thrune et al., 2009) reported a decrease when yeast culture was added to the diet. In the present study, molar proportion of acetate tended to decreased with live yeast supplementation. Decreased

acetate was also reported by Lascano and Heinrichs, (2009). The later reported that yeast supplementation increased propionate, which was not observed in the present. On the contrary, Mwenya et al., (2005) found that acetate increased and propionate decreased with yeast culture supplementation. Acetate production is mainly due to the fermentation of structural carbohydrates by cellulolytic bacteria while propionate production is mainly due to the fermentation of non-structural carbohydrates by amylolytic bacteria. The addition of yeast cultures can stimulate amylolytic bacteria that would use preferably true degradable protein.

The lack of effects of yeast on propionic acid observed in the present study agrees with previous reports in sheep (Yoon and Sten, 1996; Longusky et al., 2009) and steers (Lehloenya et al., 2008). However, the present results do not agree with Martins et al., (2000) who reported an increase in propionate concentration in vitro as a result of LY supplementation due to simultaneous conversion of lactate to propionate. This can be expected in high C:F ratio. Even at 45:55 (Besong et al., 1996) and 50:50 (Arcos-Garcia et al., 2000) C:F ratio, propionate was increased in the presence of yeast. Lascano and Heinrish, (2009) also reported an increase in propionate concentration in low and high C; F ratio. The lack of effects on propionate with LY in 60:40 C:F in the present study is unclear.

Butyrate and valerate were not affected by LY in the present study, and this is in agreement with Bargo et al., (2002), Erasmus et al., (2005) and Longusky et al., (2009) in dairy cows. The low concentration of butyrate and more escpecially valerate, iso-valerate and iso-butyrate may vary without indicating significant change.

5.2.2. Effects of monensin

Addition of M in the present study did not affect Ammonia concentration, which is uncharacteristic with monensin mode of action. These results indicate that ammonia uptake and/or de-amination of protein did not change. Monensin generally spares protein by reducing de-amination decreasing ammonia concentration in the rumen (McGuffey et al., 2001). In the present, M supplementation resulted in decreased acetate and increased propionate. Same observation was reported by Baran et al., (1986) in sheep. This indicates and energetically

efficient fermentation, as formation of propionate conserves more energy than formation of acetate (Russel and Strobel, 1989). The decrease acetate in favour of propionate resulted in an A: P ratio. The reduced ruminal production of acetate, but also butyrate (numerically) in this study is frequently attributed as the main factor reducing milk fat percentage when cows are fed diets supplemented with monensin (Van Der Werf et al., 1998).

5.2.3. Effects of live yeast and monensin

When supplemented in combination, LY and M decrease total VFA production, but did not affect Acetate concentration and only numerically reduced propionate, which resulted in decreased A:P ratio. These results suggest complementary effects of LY and M as it has been reported other studies. The decreasing effect of acetate in favour of propionate indicates more energetically efficient rumen fermentation, as observed with M supplemented alone.

5.2.4. Effects of Concentrate: Forage ratio

No effects of C:F ratio on propionate was observed in the present study, which is not in agreement with Agle et al., (2010) who indicated enhanced propionate concentration with high-grain compared with high-forage diets. Increased propionic has typically been reported in the literature (Oshio et al., 1987; Sutton et al., 2003). Moorby et al., (2006) reported increases in total VFA and butyrate concentrations and a decrease in acetate with increasing proportion of concentrate in dietary DM, but concentration of propionate was not affected. The High C diet reduced ruminal ammonia concentration, which is in agreement with.

The F:C ratio had significant effects on reducing A:P ratio when feeding monensin, showing more decrease in 60:40 diet (17.1%) diet than 40:60 diet (13.2 %). This has resulted in a tendency of increasing molar proportion of propionate when feeding M and LY+M in 60:40 diets, but not LY.

CHAPTER 6
6. CONCLUSION

Animal feed additives and rumen modifiers such as yeast cultures, probiotics, ionophores, amongst others play an essential role in alleviating metabolic disturbances in ruminants, which most often occur during early lactation. Feed additives are used as rumen manipulators to increase animal productivity. Direct fed microbial (DFM) have been used to improve animal performance. Although the results on effects of DFM are not consistent, yeast products and monensin showed varying range of benefit, which justify their continuous use in the livestock production. Many of the benefits of these additives are due to improved energy status through increased propionic acid production and a reduction in methane production.

The use of meta-analysis will continue to be a useful tool to summarize effects across studies and to investigate factors explaining potential heterogeneity of response. In the present study, the meta-analysis of monensin confirmed previous report of monensin effects, while the analysis of yeast did not.

The gas production technique evaluates cumulative gas produced as affected by treatments. The late increase in gas production with additives suggests and confirms the need of enough time for ruminal microbial population to adapt before improving digestibility of feed nutrients. Addition of monensin was more efficient when in high concentrate diet, increasing propionate at the expense of acetate. While live yeast did not affect propionate, monensin alone and the combination of monensin and live yeast increased also propionate at the expense of acetate. This particular change in VFA’s suggests more energy for production. Results of the present study indicate that these two additives will continue to play an important role in ruminant nutrition and that the effects of M and LY on rumen fermentation are substrate dependent, the high-concentrate diet showing the greatest response.

CHAPTER 7

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